Lung Toxicity Project

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Lung Toxicity Project: Categories

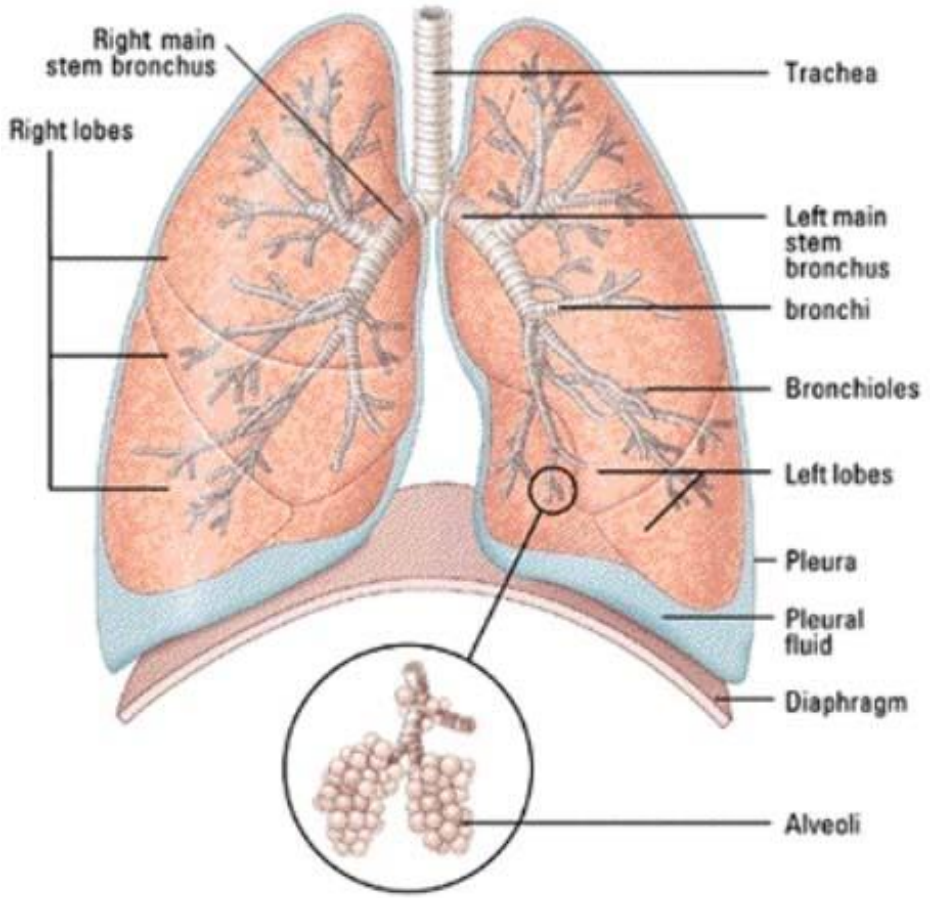
Short-term reactive process; chemicals disrupt or bind to lung membranes

- Polycationic Substances (Cationic Binding)
- General Surfactants
- Waterproofing Agents

Longer term physical process; insoluble polymers may persist in the lungs, leading to lung overload, sustained inflammation, and secondary effects

- Insoluble Polymer Lung Overload

Lung Anatomy



Lung Toxicity Project: Goals

Step 1: Develop search strategy, review abstracts, retrieve pertinent citations

Step 2: Perform study review - evaluate studies and extract POD data

Step 3: Assemble category statements and PODs (if possible)

Step 4: Provide testing strategy proposal for new chemicals

Step 5: Hazard Testing versus Exposure Mitigation Options

Category Statement Development

Category statements were developed for each category and may be considered for inclusion in the EPA New Chemical Category Document

- https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf
- Documents are available for review and include general search strategy and references

Category Statement Development

Briefing includes information from category documents:

- Chemical Class Definition
- Toxicity and Mode of Action
- Quantitative Points of Departure Tables (POD)
- Potential *In Vitro/In Chemico* Methods that may Inform Assessment
- Testing Strategy Proposals
- Hazard Testing and Exposure Mitigation Risk Management Options

Short-term process; chemicals disrupt or bind to lung membranes

- **Polycationic Substances (Cationic Binding)**
- **General Surfactants**
- **Waterproofing Agents**

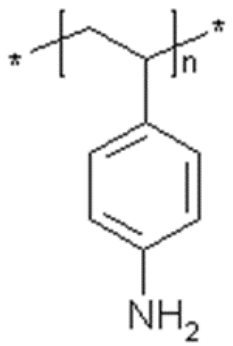
Polycationic Substances (Cationic Binding)

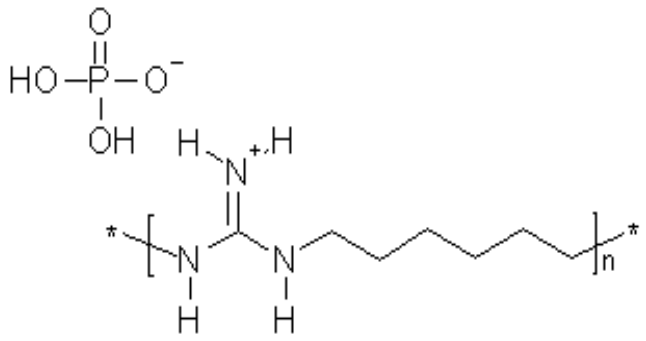
Chemical Space

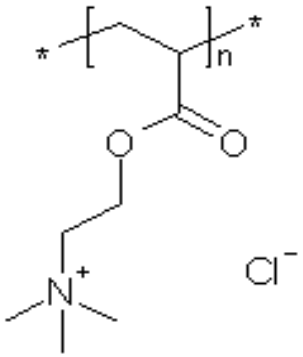
Any substance with multiple functional groups bearing positive charges at physiologically relevant pH, includes un-or partially- dissociated amines and their salts

No specific boundaries due to a paucity of data on the variety of chemicals within the space

Examples Include:







Polycationic Substances (Cationic Binding)

Toxicity and Mode of Action

- Involves electrostatic interaction with pulmonary cell membranes resulting in disruption of lipid bilayers, membrane thinning and nano-scale hole formation
- Leads to a fatal interstitial lung disease characterized by pneumonia (*i.e.*, swirls of inflammatory tissue filling the alveoli and alveolar ducts) and bronchiolitis obliterans (*i.e.*, swirls or plugs of fibrous granulation tissues filling the bronchioles)
- Cytotoxicity of category members is highly variable

Polycationic Substances (Cationic Binding)

Potential Points of Departure – Very Limited Data for Class

- Quantitative information was obtained from 2 *in vivo* inhalation exposure studies on two (2) polycationic polymers
- Studies yielded only LOAEC values
- The lowest point of departure was a LOAEC of 1.6 mg/m^3

Most conservative NCEL for category based on very limited data would be 0.0016 mg/m^3 (LOAEC/1000)

[1000 factor includes: 10x LOAEC-NOAEC extrapolation, 10x for intraspecies, and 10x for interspecies]

Polycationic Substances Abbreviated non-TSCA CBI Data Table

Chemical Name	Comments	POD value
Polyhexamethylene guanidine phosphate	rat/Sprague Dawley (sex not reported), 4 weeks exposure, 6 hours/day at 5 days/week, 2 days recovery period, 1.6 mg/m3 dose	Inflammation, metaplasia and fibrosis, LOAEC = 1.6 mg/m^3
Acramin FWR (polyurea polymer) and Acramin FWN (a polyamide-amine)	rat (strain and sex not reported), 2 weeks exposure, 6 hours/day at 5 days/week, 2 days to 2 months recovery periods, 50 or 250 mg/m3 doses	Inflammation and fibrosis, LOAEC = 50 mg/m^3
Polyhexamethylene guanidine phosphate	mouse/C57BL/7/male, saline vehicle, single dose, 14 days recovery period; 0, 0.3, 0.9, or 1.5 mg/kg doses	Fibrosis and inflammation in lung, thymus and spleen weight decreases at 0.9 mg/kg, POD NA
Acramin FWR (polyurea polymer; α,ω-diamine)	rat/Wistar/male, dilute acetic acid vehicle, Days 0 and 7 exposure; 4, 8, and 12 weeks recovery period, 0 or 0.5 mg/kg/day doses	Impaired lung function, decreased blood oxygen levels at 0.5 mg/kg POD NA
Acramin FWR (polyurea polymer) and Acramin FWN (a polyamide-amine) and Acrafix FHN (a polyamine salt)	hamster/Syrian golden/male and female, single dose, 3-92 days recovery periods; 0, 2 mg/kg Acramin FWR, or 16.5 mg/kg Acramin FWN doses	Inflammation, fibrosis, BALF enzymes increased, POD NA
Polyethylenimine (PEI) polyplex with small interfering RNA (siRNA)	mouse/BALB/c/female, single dose; 24 hours, 3 days and 7 days recovery periods, 0 or 50µL doses	LDH release in BALF, POD NA
Hydroxypropyl-β-cyclodextrin grafted PEI	rat/Sprague Dawley/male, single dose, 4 hours recovery period; 0, 2.5%, 5% or 10% w/v (100 µL); PBS Saline vehicle, Positive control was sodium deoxycholate	No effects reported, POD NA

Polycationic Substances (Cationic Binding)

In *Vitro/In Chemico* methods that may Inform Assessment

- Large body of literature related to cationic polymers as vectors for therapeutic gene delivery while reducing systemic and *in vitro* cytotoxicity in mammalian cell lines (*i.e.,* human lung A549 cells)
- *In vitro* cytotoxicity useful for evaluating mode of action for poly-cationic polymers
  - ICCVAM Recommended Protocol for the BALB/c 3T3 Neutral Red Uptake (NRU) Cytotoxicity Test - A Test for Basal Cytotoxicity
  - ICCVAM Recommended Protocol for the (human A549 cells/macrophages) Neutral Red Uptake (NRU) Cytotoxicity Test - A Test for Basal Cytotoxicity

Polycationic Substances (Cationic Binding)

Tier 1 – Use physical-chemical properties to characterize lung exposure/binding potential

- Charge density in milliEquivalents/gram or functional group equivalent weight or % amine nitrogen
- Particle Size Distribution or Aerosolized Droplet Size
  - Measurements should be activity specific (*e.g.*, chemical substance sampling at the unit operation)
  - *If respirable (i.e., ≤ 10 microns) during manufacturing, processing, or use, then proceed to Tier II. If not respirable, determine if Tier II testing is needed*

Polycationic Substances (Cationic Binding)

Tier II- Proposed *In Vivo* Studies**

- Step 1: OECD Acute TG 403 featuring rats exposed for 4 hours and observed for 2 weeks (LOAEC < 2000 mg/m^3, proceed to step 2)

- Step 2: 5-day study to address toxicity progression (substantial decrease in the POD over time relative to the acute study, proceed to step 3)

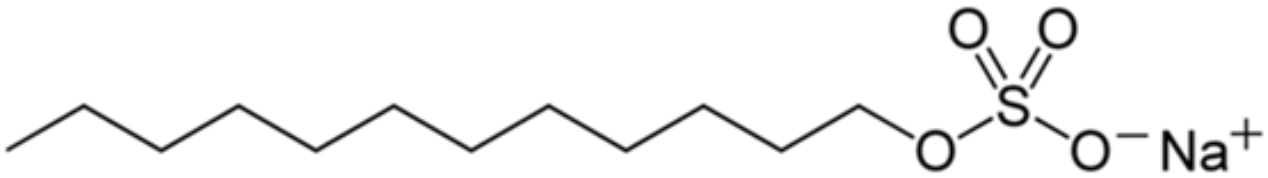
- Step 3: OECD TG 412 (28-day inhalation study in rats with 14-day recovery period)

*** Possible modifications to all above studies include pulmonary function testing, analysis of BALF, LDH release and blood O_2 content, and satellite reversibility*

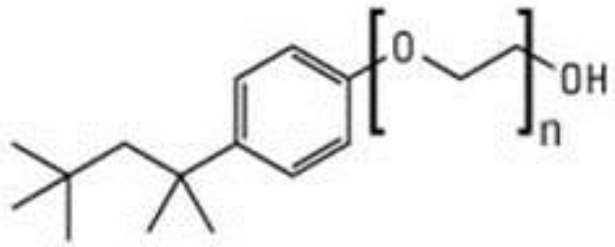
General Surfactants: Ionic, Cationic, & Non-Ionic

Chemical Space

- Includes anionic, cationic, and nonionic surfactants
- No specific boundaries due to a paucity of data on the variety of chemicals within the space
- Examples include:



Sodium dodecyl sulfate



Triton* X-100 Detergent
n = 9-10
MW 647

General Surfactants

Toxicity and Mode of Action

- Interfere with natural surfactants, resulting in decreased oxygen uptake
- Dysfunction of natural surfactant caused increased alveolar permeability
- Other pulmonary effects included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (*i.e.*, collapsed alveoli).
- Can cause membrane disruption

General Surfactants

Potential Points of Departure – Very Limited Data for Class

- Quantitative information was obtained from 13 published reports of *in vivo* inhalation exposure on four (4) chemicals
- None of the studies reported the data in a way to identify NOAEC/LOAEC values for inhalation exposure
- Additional 21-day study from submitter on related chemical didecyl dimethyl ammonium chloride (DDAC) was identified by EPA as a potential POD
  - Resulting LOAEC of 0.08 mg/m^3 may represent multiple effects (not surfactant effect alone)

Most conservative NCEL for the category based on DDAC data would be 0.00008 mg/m^3 (LOAEC/1000)

[1000 factor includes: 10x LOAEC-NOAEC extrapolation, 10x for intraspecies, and 10x for interspecies]

General Surfactants Abbreviated non-TSCA CBI Data Table

Chemical Name	Comments	POD value
Defomaire (superinone respiratory detergent containing silicone)	Human volunteers (healthy, trained), 6 minute duration, 3 mL dose, water vehicle	ND
Alevaire (tyloxapol)	Dog, Greyhound, sex NS, 8 hour duration, saline vehicle	ND
Triton X-100 (polyethylene glycol p-isooctylphenyl ether)	Hamster, Syrian, male and female, 95 days old, 37 minute duration, Ethanol solvent, lung burdens of 800, 1400, 1900, 2500 or 3100 µg, 7 days recovery period	LD50 = 1700 µg 3000 mg/m3
Triton X-100	Hamster, Syrian, male and female, 419 days old, 37 minute duration, Ethanol solvent, lung burdens of 800, 1400, 1900, 2500 or 3100 µg, 7 days recovery period	LD50 = 1700 µg 3000 mg/m3
Triton X-100	Hamster, Syrian, male and female, two consecutive lavages, 374 days old, saline vehicle, 7 day recovery period, 0, 0.01%, 0.05%, 0.06%, 0.075% or 0.10% in saline	ND
Dioctyl sodium sulfosuccinate (DOSS); Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	Dog, mongrel, sex NS, adult, 35-45 minute duration, 15 mg/kg dose, 2 hour recovery period, ethanol/saline vehicle,	ND
Dioctyl sodium sulfosuccinate (DOSS)	Dog, mongrel, sex NS, adult, 35-45 minute duration, 15 mg/kg dose, 2 hour recovery period, ethanol/saline vehicle,	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, 5 minute exposure, 60 minute recovery period, 1% solution in ethanol/saline	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, 5 minute exposure, 30 minute recovery period, ethanol and saline vehicle, 1% solution of DOSS	ND
Dioctyl sodium sulfosuccinate (DOSS)	Sheep, mixed-breed, F, adult, 1 hour exposure period, 24 hour recovery period, ethanol and saline vehicle, 15 mg/kg dose	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, 5 minute exposure period, 3 hour recovery period, 0.125% - 2% solution in ethanol and saline vehicle	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, Duration not known, 0 or 10 µL of 2% detergent solution in ethanol and saline deposited in pulmonary parenchyma, 3 hour recovery period	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, Duration not known, 0 or 10 µL of 2% detergent solution in ethanol and saline deposited in pulmonary parenchyma, 3 hour recovery period	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, 5 minute duration, 0 or 10 µL of 2% detergent solution in ethanol and saline deposited in pulmonary parenchyma, 3 hour recovery period	ND
Dioctyl sodium sulfosuccinate (DOSS)	Rabbit, strain and sex NS, Duration not known, 0 or 10 µL of 2% detergent solution in ethanol and saline deposited in pulmonary parenchyma, 3 hour recovery period	ND
Didecyl Dimethyl Ammonium Chloride (DDAC)	Rats, 21 day study, 6 hours/day, 5 days/week	Increased lung weight, inflammation, LDH release, hemorrhage, LOEAC = 0.08 mg/m3

General Surfactants

***In Vitro/In Chemico* methods that may Inform Assessment**

- Surface Tension measurements using *c*apillary surfactometer to evaluate effect of chemicals on function of bovine-derived surfactant *in vitro*
- Biosolubility Measurements – Described in the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Technical Report 122 Section 3
- *In vitro* cytotoxicity assay using human lung A549 epithelial cells
- *In chemico* surface tension measurements relative to DDAC and SDS inform toxicity potency.
- *In vitro* results were highly predictive of *in vivo* toxicity, but do not by themselves constitute adequate tests for acute pulmonary toxicity

General Surfactants

Tier 1 – Use physical-chemical properties to characterize lung exposure/disruption

- Particle Size Distribution or Aerosolized Droplet Size
- Surface Tension Decreases (capillary surfactometer/bovine-derived lung surfactant *in vitro)*
  - Measurements should be activity specific (*e.g.*, chemical substance sampling at the unit operation)
  - *If respirable (i.e., ≤ 10 microns) during manufacturing, processing, or use, and surface tension decreases, then proceed to Tier II. If not respirable, determine if Tier II testing is needed.*

General Surfactants

Tier II- Proposed *In Vivo* Studies

- Step 1: OECD Acute TG 403 featuring rats exposed for 4 hours and observed for 2 weeks (LOAEC < 2000 mg/m^3, proceed to step 2)

- Step 2: 5-day study to address toxicity progression (substantial decrease in the POD over time relative to the acute study, proceed to step 3)

- Step 3: OECD TG 412 (28-day inhalation study in rats with 14-day recovery period)

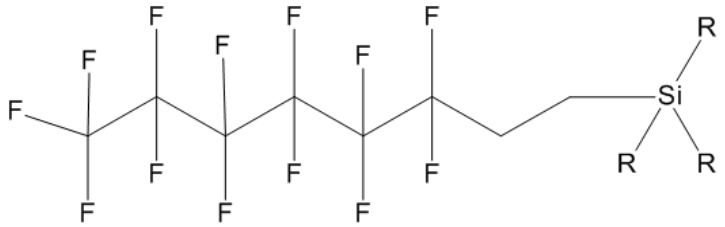
*** Possible modifications to all above studies include pulmonary function testing, analysis of BALF, LDH release and blood O_2 content, and satellite reversibility*

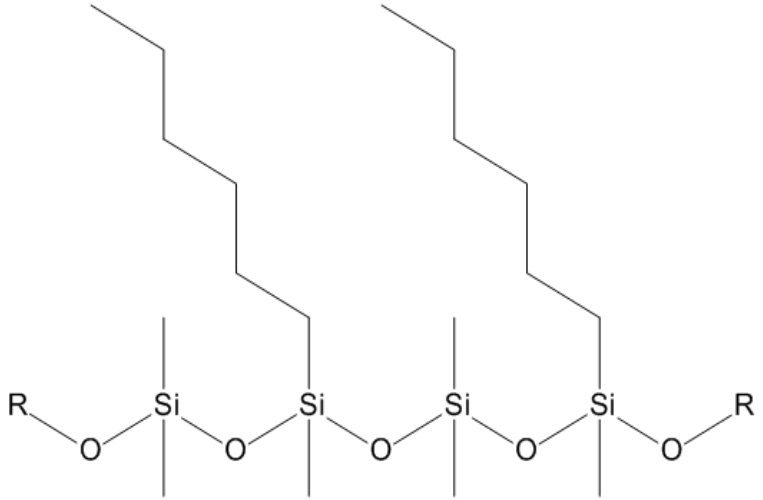
Waterproofing Agents

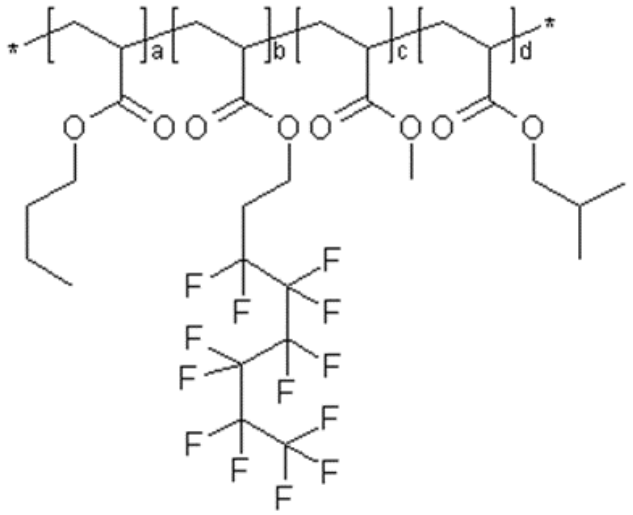
Chemical Space

Compounds to repel water or stains (perfluros, alkoxy silanes): Applied to a wide variety of solid surfaces

Examples include:







Waterproofing Agents

Toxicity and Mode of Action

- Respiratory symptoms such as cough, shortness of breath, and chest pain may occur within minutes of exposure
- Interfere with natural surfactants in lung: Surface tension at alveoli increases, oxygen transfer decreases, resulting in decreased blood oxygen
- May progress in severe cases to pulmonary edema and hemorrhage, diffuse pulmonary collapse, respiratory failure, and death

Waterproofing Agents

Potential Points of Departure – Very Limited Data for Class

- Quantitative information was obtained from 13 published reports of *in vivo* inhalation exposure on >50 product formulations
- 9 of the studies yielded NOAEC/LOAEC values, some for multiple product formulations
- Not all values are directly comparable due to methodological differences across studies
- Effect levels vary widely based on how exposure concentrations were determined and influenced by aerosol droplet size and solvent present
- The lowest reported point of departure was a LOAEC of 1.5 mg/m^3

Most conservative NCEL for the category based on very limited data would be 0.0015 mg/m^3 (LOEAC/1000)

[1000 factor includes: 10x LOAEC-NOAEC extrapolation, 10x for intraspecies, and 10x for interspecies]

Waterproofing Agents Abbreviated non-TSCA CBI Data Table

Chemical Name	Comments	POD value
Commercial product (WetGuard, Nichiban Co., Tokyo Japan) containing fluororesin and silicone resin as water-repellent, ethyl acetate, mineral turpentine and n-heptane as solvent and propane as propellant	Mouse, ICR CD-1, female; 95 minute duration; solvents present	Cyanosis, hemorrhage, POD ND
Fluororesin spray (ST395, NOK Co., Tokyo Japan) containing fluororesin as water-repellent, ethyl acetate as solvent and butane/propane as propellant	Mouse, ICR CD-1, female; 95 minute duration, ethyl acetate solvent	Cyanotic, hemorrhage, POD ND
Silicone resin spray (SD8000) containing silicone resin as water-repellent, mineral turpentine as solvent and butane/propane as propellant	Mouse, ICR CD-1, female, 95 minute duration;	POD ND
Twelve commercial waterproofing sprays containing fluorocarbon resins of unknown structure, grouped according to reported acute respiratory effects in humans into a Toxic Group (Sprays #1-4) and a Non-Toxic Group (Sprays #5-12)	Mouse, ICR CD-1, female; 95 minute duration;	Alveolar collapse, hemorrhage, POD ND
Four waterproofing sprays (A, B, C, D) containing 9.6 g of fluorocarbon resin (ST395, NOK Co., Tokyo Japan), 228 g of n-heptane, 2.4 g of ethyl acetate and 60 g of liquified petroluem gas, differing only in propane:butane ratio of the LPG to make different mist particle sizes.	Mouse, ICR CD-1, female; 95 minute duration;	Alveolar collapse, POD ND
Commercial leather conditioner (newer formulation identified as 1292, associated with a 1992 outbreak of human respiratory disease)	Rat, Sprague-Dawley, male; varying durations; 8 hour recovery period; 0.5 hr (1.5 mg/m3), 1.5 hr (1.9 mg/m3), or 2 hr (3.2 mg/m3)	Edema, death, rapid breathing, necrosis, hemhorrage, LOAEC = 1.5 mg/m3
Commercial leather conditioner (newer formulation identified as 1292, associated with a 1992 outbreak of human respiratory disease)	Guinea Pig, English shorthair, male; 2 hour duration; 18 hour recovery period; 0 , 1.5 or 3.2 mg/m3 doses	Edema, death, rapid breathing, necrosis, hemorrhage, LOAEC = 1.5 mg/m3
Commercial leather conditioner (older formulation identified as 1092)	Guinea Pig, English shorthair, male; 2 hour duration; 18 hour recovery period; 0 or 3.3 mg/m3 doses	Macrophage increase in BAL fluid, NOAEC = 3.3 mg/m3
Experimental fabric protector consisting of fluororesin (perfluoro alkylethyl acrylate/n-alkyl acrylate copolymer, 1.0% by weight) and organic solvents naphtha (95.0%), heptane (3.0%), and ethyl acetate (1.0%)	Rat, Wistar, adult; 5 breaths duration; 9 hour recovery period; 480,000 mg/m3	Decreased oxygen levels that were increased by lung surfactant add back, LOAEC = 480,000 mg/m3
The test articles were Magic Nano Glass & Ceramic [GC], Magic Nano Bath [B], and pump spray [P]. The concentration of silanes was less than 1%.	Rat, Wistar, male and female; 4 hour duration; 2 week recovery period; 0, 8.9, 9.3, 33.0, 120.4 [GC], 85.2 [B], and 1857 mg/m3 doses	Irregular breathing, death, decreased temperature, LOAEC = 2269 mg/m3
Nanospray film product used as floor sealant (NFP-1), contained 2-propanol (solvent) and unspecified fluorosilane.	Mouse, BALB/cA, male; 60 minute duration; 24 hour recovery period; NFP-1: 0, 3.3, 15.7, 16.1, 18.4, 24.4 or 42.4 mg/m3; Synthetic NFP: 46.3 mg/m3 perfluorosilane: 1.8 mg/m3; perfluorodisiloxane: 26.5 mg/m3; propanol or ethanol solvent	NOAEC = 16.1 mg/m3 for respiratory effects (per study authors) NOAEC = 3.3 mg/m3 overall based on body weight

Waterproofing Agents Abbreviated non-TSCA CBI Data Table

Chemical Name	Comments	POD
Nanospray film product used for coating of ceramic tiles (NFP 2), contains ethanol and methanol (solvents) and unspecified alkylsilane.	Mouse, BALB/cA, male; 60 minute duration; 24 hour recovery period; ethanol solvent; NFP-2: 0, 33.2 or 60.0 mg/m3 doses	Reduced tidal volume, NOAEC = 33.2 mg/m3
Seven commercially available water-based NFPs were included in this study, all contained hydrolyzed forms of 1H,1H,2H,2H-perfluorooctyl trialkoxysilane (POTS).	Mouse, BALB/cA, male; 60 minute duration; 15 minute recovery period; methanol and propanol solvents; 12 (NFP), 39-51 (various POTS-solvent formulations), or 110 (NFP concentrate) mg POTS/m3	Reduced tidal volume, LOAEC = 39 mg/m3
Nanofilm product (NFP) obtained from NanoCover containing hydrolysates and condensates (siloxanes) of perfluorooctyl triisopropoxysilane dissolved in 2-propanol	Mouse, BALB/cA, male; 60 minute duration; 30 minute recovery period; propanol solvent; 0 or 18.4 mg/m3 doses	Increased airway resistance, LOAEC = 18.4 mg/m3
Commercial tile-coating product Stain Repellent Super (SRS, Akemi GmbH, Nürnberg, Germany). The chemical analysis by MS showed presence of alkylsiloxanes and naptha and C9–C13 alkanes	Mouse, BALB/cA, male; duration 10-60 minutes; 30 minute recovery period; 59, 76, 103, 304 or 5700 mg/m3 doses	Reduced tidal volume, intoxication, NOAEC = 59 mg/m3
Commercial tile-coating product Stain Repellent Super (SRS, Akemi GmbH, Nürnberg, Germany), presence of alkylsiloxanes and naptha and C9–C13 alkanes	Human (39 cases resulting from single episode); 10-150 minutes duration; doses of 563 mg/m3 immediately after the spraying, to 438 mg/m3 1 h later and 184 and 34 mg/m3 after 3 and 19 h, respectively	Chest pain, coughing, decreased oxygen levels, increased temperature, POD ND
RapiAquaStop (Werner & Mertz GmbH, Mainz, Germany) was the most frequently involved spray (46% of cases). The two other sprays reported were K2R(K2R Produkte GmbH, Gottmadingen, Germany) and RapiIntemp (Werner & Mertz). —a mixture of fluorinated acrylate polymer and isoparaffinic hydrocarbons.	Human (102 cases in Switzerland between Oct 2002 and March 2003); spraying times up to 90 minutes and residence time up to 12 hours; Simulated maximal exposure concentrations ranged from 0.003 - 35.98 mg/m3 (mean = 4.21 mg/m3) and estimated doses up to 11.27 mg (mean value = 0.657 mg)	Cough, nausea, POD ND
NanoCover (Aalborg, Denmark): “Non-absorbing floor materials” NAFM, [perfluorsilan/siloxan (POTS) in 2-propanol], “Textiles and leather” [perfluorsilan/siloxan in water], “Textiles and leather concentrate” [perfluorsilan/siloxan in water], “Bath and tiles” [alkylsilan/siloxan in ethanol] and “Car glass” [alkylsilan/siloxan in ethanol]. The product “Footwear protector” [perfluoroacrylate in water and glycoethers] “Special textile coating” [perfluorsilan/siloxan in water] and “Rim sealer” [perfluorsilan/siloxan in mixture of 2-propanol, 1-methoxy-2-propanol and ethylacetate]. “Wood impregnation” [perfluoroacrylate in water and glycoethers.	Mouse, BALB/cA, male; Up to 60 minute duration; 15 minute recovery period;	NOAEC = 6 mg/m3 [FP] NOAEC = 33 mg/m3 [WI], NOAEC = 2958 mg/m3 [NAFM]

Waterproofing Agents

***In Vitro/In Chemico* methods that may Inform Assessment**

- Capillary surfactometer to evaluate effect of waterproofing sprays on function of bovine- derived surfactant *in vitro*
- Biosolubility Testing - ECETOC TR122
- *In vitro* cytotoxicity assay using human lung A549 epithelial cells
- *In vitro* results were highly predictive of *in vivo* toxicity, do not by themselves constitute adequate tests for acute pulmonary toxicity

Waterproofing Agents

Tier 1 – Use physical-chemical properties to characterize lung exposure/disruption

- Particle Size Distribution or Aerosolized Droplet Size
- Surface Tension Increases (capillary surfactometer/bovine-derived lung surfactant *in vitro)*
  - Measurements should be activity specific (*e.g.*, chemical substance sampling at the unit operation)
  - *If respirable (i.e., ≤ 10 microns) during manufacturing, processing, or use, and surface tension increases, then proceed to Tier II. If not respirable, determine if Tier II testing is needed.*

Waterproofing Agents

Tier II- Proposed *In Vivo* Studies **

- Step 1: OECD Acute TG 403 featuring rats exposed for 4 hours and observed for 2 weeks (LOAEC < 2000 mg/m^3, proceed to step 2)

- Step 2: 5-day study to address toxicity progression (substantial decrease in the POD over time relative to the acute study, proceed to step 3)

- Step 3: OECD TG 412 (28-day inhalation study in rats with 14-day recovery period)

*** Possible modifications to all above studies include pulmonary function testing, analysis of BALF, LDH release and blood O_2 content, and satellite reversibility*

Longer term physical process; lung overload, inflammation, and secondary effects

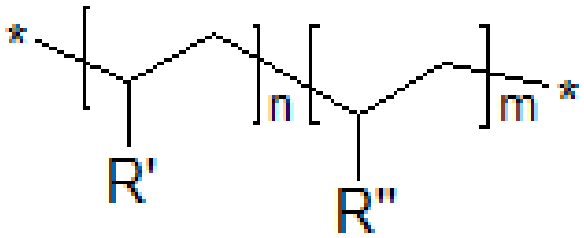
- **Insoluble Polymer Lung Overload**

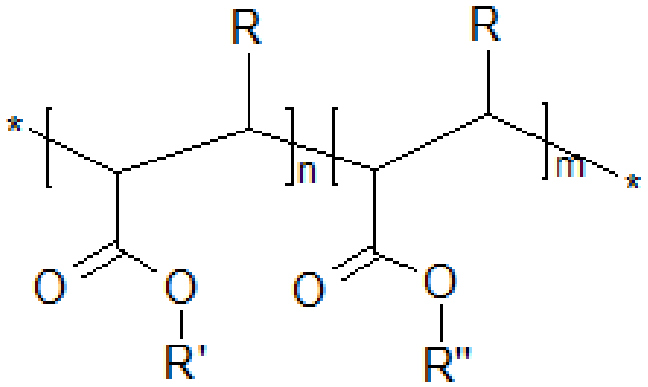
Insoluble Polymer Lung Overload

Chemical Space

Insoluble, respirable polymers: Polyacrylates, Polyvinyls, *etc*.

- Large number of materials could lead to very broad sub-category definitions
- No specific boundaries due to a paucity of data on the variety of chemicals within the space
- Includes common materials such as polystyrene and PVC





Insoluble Polymer Lung Overload

Toxicity and Mode of Action

- Insoluble polymers may persist in the lungs: physical, non reactive process, but may lead to lung overload, sustained inflammatory response, and secondary effects
  - Particles less than 10 microns are assumed to enter the deep lungs
- Effects from sustained inflammation due to long-term inhalation exposure to concentrations producing high lung burdens range

Insoluble Polymer Lung Overload

Potential Points of Departure – Very Limited Data for Class

- Quantitative information was obtained from four (4) *in vivo* inhalation exposure studies on two (2) poorly soluble polymers
- All four (4) of the studies yielded NOAEC/LOAEC values
- The lowest reported point of departure was a LOAEC of 3.3 mg/m^3

Most conservative NCEL for the category based on very limited data would be 0.0033 mg/m^3 (LOAEC/1000)

[1000 factor includes: 10x LOAEC-NOAEC extrapolation, 10x for intraspecies, and 10x for interspecies]

Polymer Lung Overload Abbreviated non-TSCA CBI Data Table

Chemical Name	Comments	POD value
A 9000-type xerographic toner material composed of about 90% 58:42 styrene/l-butylmethacrylate random copolymer (CAS no. 25213-39-2) and 10% high-purity furnace-type carbon black (CAS no. 7440-44-0) was specially prepared, the respirable fraction of particles was enriched about 10-fold. 70,000 Da	Rat, SPF F-344, male and female; 3 months duration, 6 hours/day at 5 days/week; 3 month recovery period; Air control; 0, 1, 4, 16 or 64 mg/m3 doses	Lung weight increase, Reduced clearance, Lung hyperplasia & hypertrophy NOAEC = 4 mg/m3
A 9000-type xerographic toner material composed of about 90% 58:42 styrene/l-butylmethacrylate random copolymer (CAS no. 25213-39-2) and 10% high-purity furnace-type carbon black (CAS no. 7440-44-0) was specially prepared for animal studies.	Rat, SPF F-344, male and female; 24 months duration, 6 hours/day at 5 days/week; 2 months recovery period; 0, 1, 4, or 16 mg/m3 doses; air exposed control; also TiO2 as negative and chrystalline SiO2 as positive controls	Lung fibrosis, decreased alveolar clearance, NOAEC = 1 mg/m3
A 9000-type xerographic toner material composed of about 90% 58:42 styrene/l-butylmethacrylate random copolymer (CAS no. 25213-39-2) and 10% high-purity furnace-type carbon black (CAS no. 7440-44-0) was specially prepared for animal studies.	Rat, SPF F-344, female; 3 months duration, 6 hours/day at 5 days/week; 15 months recovery period; air exposed control group; 0, 10, or 40 mg/m3	LDH release, decreased alveolar clearance, LOAEC = 10 mg/m3
polyvinyl chloride (PVC) powder	Rat, strain NS, female; 7 months duration at 25 hours/week; recovery period 100 days; air exposed control group; 0, 3.3, 8.3 or 20.2 mg/m3 doses	Decreased alveolar clearance, LOAEC = 3.3 mg/m3
polystyrene spheres	Rat, strain NS, sex NS; polymer instilled in airway; 6 months recovery period;	Only 3 micron particles were cleared from the lungs, POD ND
Poorly soluble, slowly biodegradable linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da	Rat, SPF Wistar (sex NS); 4 hour duration; 14 day recovery period; 910 mg/m3 dose	Labored breathing, hypothermia, LOAEC = 910 mg/m3
Poorly soluble, slowly biodegradable linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da	Rat, SPF Wistar, male; 6 hour duration; 7 day recovery period; water vehicle; 0, 57 or 979 mg/m3 doses	LDH release, hypothermia, NOAEC = 57 mg/m3
Poorly soluble, slowly biodegradable linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da	Rat, SPF Wistar, male; 2 week duration at 6 hours/day at 5 days/week; 2 week recovery period; water vehicle; 0, 5, 22 or 121 mg/m3 doses	LDH release, macrophage inclusions, inflammation, hypercellularity, NOAEC = 22 mg/m3
Poorly soluble, slowly biodegradable linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da incorporating both hydrophilic and hydrophobic segments, the insoluble content of dispersion was approximately 30%.	Rat, SPF Wistar, male and female; 13 week duration at 6 hours/day at 5 days/week; 4 week recovery period; water vehicle; 0, 5, 26 or 107 mg/m3 doses	LDH release, macrophage inclusions, hypercellularity, NOAEC = 5 mg/m3
Aqueous dispersion resin (ADR) is a water-based acrylate copolymer supplied by Amerchol Corporation (lot 10-19-92; Edison, NJ) containing 26% of an acrylic latex consisting of ethyl acrylate, methacrylic acid, methyl methacrylate, acrylic acid polymer (CAS RN 25053-63-8), formulated in 73% water neutralized to pH 7 with 1% salts and surfactants.	Rat, Sprague-Dawley, male and female; 13 week duration, 2 hours/day at 5 days/week; 6 week recovery period; air control group; 0, 30, 100 or 300 mg/m3 doses	Lung weight increase, inflammation, macrophage accumulation, NOAEC = 30 mg/m3
Butyl acrylate/methacrylic acid polymer diluted in ethanol	Rat (no further data); 13 weeks duration; 6 week recovery period; Ethanol solvent; 0, 1, 10 or 30 mg/m3 doses	Macrophage accumulation, NOAEC = 10 mg/m3

Polymer Lung Overload

***In Vitro/In Chemico* methods that may Inform Assessment**

- The mode of action for pulmonary toxicity and carcinogenicity of poorly soluble polymers involves impairment of alveolar macrophage-mediated clearance of particulates from the lungs.
- An *in vitro* assay using NR8383 cells/A549 derived from rat alveolar macrophages differentiate biologically active particulates with specific toxicity
  - Testing currently limited to nanoparticles
  - *In vitro* results accurately predicted toxicity of the 20 nanoparticles
  - Bio-Solubility Test using mammalian fluids

Polymer Lung Overload

Tier 1 – Use physical-chemical properties to characterize lung exposure

- Particle Size Distribution or Aerosolized Droplet Size
- Biosolubility – Described in ECETOC Technical Report 122 Section 3
  - Measurements should be activity specific (*e.g.*, chemical substance sampling at the unit operation)
  - *If respirable (i.e., ≤ 10 microns) during manufacturing, processing, or use and poorly soluble, then proceed to Tier II. If not respirable, determine if Tier II testing is needed.*

Polymer Lung Overload

Tier II- Proposed *In Vivo* Studies**

- Step 1: OECD Acute TG 403 featuring rats exposed for 4 hours and observed for 2 weeks (if test substance is retained in the lung, proceed to step 2)
- Step 2: 5-Day study to evaluate lung burden and to inform pulmonary deposition and retention of particles in the lung (multiple post-exposure sacrifices that demonstrate lung burden over time, proceed to step 3)
- Step 3: OECD TG 412 to evaluate lung burden, clearance, and translocation (multiple post-exposure sacrifices that demonstrate lung burden by decreased lung clearance kinetics over time (28-day inhalation study in rats with 14-day recovery period)

Polymer Lung Overload

- Step 4: OECD TG 413 to evaluate lung burden, clearance, and translocation (multiple post-exposure sacrifices that demonstrate lung burden by decreased lung clearance kinetics over time (90-day inhalation study in rats with 60-day recovery period).
- If the results of the subchronic 90-day study indicate particles have carcinogenic potential (*e.g.*, sustained inflammation), then proceed to Tier III.

Tier III – Proposed *In Vivo* Studies**

- A 2-year inhalation bioassay in rats may be warranted (exposure concentration high enough to impair pulmonary clearance of particles and lead to an "overload" condition).

** Possible modifications to all above studies include special attention to pulmonary function tests; lung burden measurements and lung clearance kinetics; collection of bronchoalveolar lavage fluid (BALF) for assessment of marker enzyme activities, total protein content, and cell counts; lung retention and clearance; lung weight; and lung histopathology (inflammation and cell proliferation). It is not necessary to look at internal organs.

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